

Aging and Innate Immunity

Commentary

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Adaptive immunity undergoes severe deterioration with age and represents the main problem in the elderly. However, evidence accumulated over the last decade supports the hypothesis that aging also has a profound impact on innate immunity, which in turn markedly impacts the health and longevity of older people.

The aged immune system is less able to mount an effective immune response after challenges with infectious pathogens than the young because of complex changes collectively termed “immunosenescence.” This condition has been demonstrated in diverse species including humans and contributes to morbidity and mortality due to the greater incidence or reactivation of infectious diseases, as well as the possible enhanced susceptibility to autoimmune diseases and cancer (Pawelec et al., 2002).

Human aging is generally accompanied by elevated systemic inflammatory conditions. Thus, inflammation-related biomarkers are powerful predictors of frailty and mortality in the elderly (DeMartinis et al., 2006), and this phenomenon is referred to as “inflamm-aging” (Franceschi et al., 2000). In aged subjects, the alterations in the production of inflammatory mediators might be caused by preexisting conditions such as autoimmune or degenerative diseases, cancer, or other factors that diminish the ability to combat infections. However, they may also result from age-associated intrinsic defects in the innate immune system, one of the major functions of which is to maintain the cytokine balance and control inflammation.

Studies in the immune system in elderly humans and old mice have clearly demonstrated that adaptive immunity suffers a severe deterioration with age, likely due to a combination of intrinsic and extrinsic factors (see Weng, 2006, in this issue of *Immunity*). Thus, whereas thymic involution, presumed to be intrinsic, is one of the main causes of the well-accepted decrease in num-

bers of naive T cells in the elderly, persistent life-long antigenic stress from immunosurveillance against persistent viruses, especially cytomegalovirus (CMV), is responsible for the accumulation of effector-memory CD8⁺ T cells and the marked reduction of the T cell repertoire observed with age (Pawelec et al., 2005; Tarazona et al., 2000). In contrast, in the complex scenario of immunosenescence, it had been generally accepted that some aspects of innate immunity, e.g., phagocytosis and natural killer (NK) cell cytotoxicity, remain largely unaffected (Pawelec et al., 1998). However, a more detailed analysis has demonstrated a profound impact of aging on cells involved in the innate immune response. Cellular components of the innate immune system, including neutrophils and macrophages, are the first to arrive at the site of infection. Their role is to initiate an inflammatory response, phagocytose the pathogen, recruit NK cells, and facilitate the maturation and migration of dendritic cells (DCs) that will regulate and determine the nature of the T cell-mediated outcome. Thus, alterations in innate immunity in the elderly have great importance for pathogen resistance. Here we discuss the hitherto underappreciated impact of age-associated changes in the innate immune system.

Phagocytosis and Elimination of Pathogens

Phagocytes play an essential role during the first phase of the immune response against pathogens and are major constituents of innate immunity, contributing to inflammatory responses. Innate immune recognition of pathogens relies on a set of germline-encoded pattern recognition receptors (PRRs) that recognize conserved molecular structures related to microorganisms (also known as pathogen-associated molecular patterns). The best characterized of these receptors are the Toll-like receptors (TLRs) (Akira et al., 2006). Ligand of TLRs on neutrophils enhances phagocytosis of pathogens and release of antimicrobial peptides, and through the production of chemokines, it recruits and activates other immune cells at the site of the infection. Although the elderly preserve the number and phagocytic capacity of neutrophils, other functional characteristics of these cells are altered during aging (Figure 1). For example, receptor-driven functions such as superoxide anion production, chemotaxis, and apoptosis are reduced. A decrease in signaling elicited by specific receptors and alterations in the neutrophil membrane lipid rafts are involved in the defective function of neutrophils with advancing age (Fulop et al., 2004). In particular, although the expression of receptors such as TLRs and GM-CSF receptor is not diminished, their ligation results in altered signal transduction, possibly as a consequence of changes to the structure, dynamics, and function of lipid rafts (Fulop et al., 2004). In young individuals, lipopolysaccharide (LPS) stimulation induces the recruitment of TLR4 in both membrane raft and nonraft fractions. In contrast, in the elderly, LPS stimulation does not induce changes in the recruitment of TLR4 into the lipid rafts (Fulop et al., 2004). In addition, activation through GM-CSF receptor is altered in the elderly. Whereas in young individuals the binding of GM-CSF

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Neutrophil	Macrophage	Dendritic cell	NK cell
			
Preserved Number Phagocytosis TLR2, TLR4 expression GM-CSFR expression	Number	Antigen presentation (decreased in frail elderly)	Overall cytotoxicity (decreased in frail elderly) CD16-mediated cytotoxicity
Reduced Superoxide production Chemotaxis Apoptosis Signal transduction Molecule recruitment into lipid rafts	Phagocytosis Superoxide production Chemotaxis Apoptosis TLR expression (?) Signal transduction Cytokine production MHC class II expression	Number of Langerhans cells Langerhans cell migration	Per cell cytotoxicity Signal transduction Response to cytokines Cytokine production
Increased	PGE2 production		Number of CD16 ⁺ CD56 ^{dim} (decreased in frail elderly)

Figure 1. Effects of Aging on Cellular Components of the Innate Immune System

to its receptor induces the displacement of Src homology (SH) domain-containing protein tyrosine phosphatase-1 (SHP-1) from the lipid rafts, in the elderly, this phosphatase is not downregulated (Fortin et al., 2006). Because the function of SHP-1 is to dephosphorylate proteins involved in activation pathways, the presence of the phosphatase in the lipid raft would block cell activation, contributing to the decreased response to GM-CSF observed in neutrophils from elderly individuals.

The phagocytic function of macrophages is also impaired with age (Figure 1). Macrophages are a heterogeneous group of phagocytes present in most tissues that are implicated in inflammatory and immunological responses. Thus, macrophages play an essential role as sensors of exogenous and endogenous danger signals through pattern recognition receptors including TLRs. Although the number of monocytes in peripheral blood does not change substantially with age, decreased number of macrophage precursors and bone marrow macrophages has been observed in humans (Plowden et al., 2004). Moreover, in elderly individuals, the phagocytic ability of macrophages decreases in parallel with reduced production of macrophage-derived cytokines (Plowden et al., 2004). However, endocytic receptors involved in the phagocytic process and their signal transduction have not yet been analyzed in relation to aging. Similar to neutrophils, aging also affects the production of superoxide anion and nitrous oxide. Altered responses to chemotactic stimuli and decreased chemokine production have also been described (Plowden et al., 2004; Gomez et al., 2005). The analysis of TLR expression in macrophages from aged mice has so far yielded somewhat discrepant results. In one report, no changes were found in the expression of TLR2 and TLR4 on splenic macrophages analyzed by flow cytometry, but another study showed decreased expression of TLR1 through TLR9. However, both studies agreed that defects in TLR function were present (Boehmer et al., 2005; Plowden et al., 2004). Altered signal transduction

pathways could be involved in the defective production of cytokines by aged macrophages after TLR stimulation (Gomez et al., 2005; Boehmer et al., 2005). Together, these alterations in neutrophil and macrophage functions and signaling pathways associated with aging may be responsible for the changes observed in phagocyte functions and contribute to the substantial increase in infections in old age.

Antigen Presentation

Macrophages express MHC class II molecules and are involved in the initiation of the adaptive immune response against pathogens by acting as antigen-presenting cells (APCs). Antigen presentation by macrophages is decreased with age, possibly due to diminished expression of MHC class II molecules both in humans and mice (Plowden et al., 2004; Herrero et al., 2001). In addition, the induction of the MHC class II gene (*H2-Ab1*) in response to IFN- γ is impaired in macrophages from aged mice due to the decreased binding of transcription factors to the W and X boxes of the promoter (Herrero et al., 2001). Moreover, activated macrophages from aged humans and mice produce higher amounts of prostaglandin E2 than younger individuals, which inhibits surface expression of MHC class II, thus contributing to the decreased capacity of antigen presentation of macrophages observed with age (Plowden et al., 2004).

Dendritic cells (DCs) are the primary APCs, and activation of DCs through PRRs such as TLRs induces their maturation and migration to secondary lymphoid organs where they present antigens to T cells and initiate an immune response. Most studies on age-associated changes in the phenotype and function of DCs have analyzed the characteristics of Langerhans cells (LCs) in the skin (Figure 1). A decrease in the number of these cells has been described in elderly people and old mice. However, it is not clear whether this defect is a consequence of diminished bone marrow precursor production. LC migration to lymph nodes is also decreased in aged mice. This defect may be secondary to

a reduced availability of IL-1 β because LC-derived IL-1 β provides an autocrine stimulus for migration and provokes the production of TNF- α by adjacent keratinocytes, which also activates LC mobilization (Cumberbatch et al., 2002).

In contrast to macrophages, DCs from healthy elderly people seem to retain their capacity to efficiently present antigen to T cells, suggesting that age-related decline of T cell function is not the consequence of altered antigen presentation (Lung et al., 2000). However, DCs from frail elderly people may have decreased expression of costimulatory molecules and IL-12 production and therefore impaired ability to induce T cell proliferation. It has been hypothesized that in the healthy elderly, age-related changes in the T cell compartment can be compensated by a boost in DC function (Uyemura et al., 2002). In addition, the production of IL-10 is elevated in elderly individuals and may inhibit DC maturation and macrophage function (Uyemura et al., 2002). Overall, the exact picture regarding DC dysfunction with aging is still emerging.

NK Cell Cytotoxicity as a Biomarker of Healthy Aging

NK cells are one of the cellular mediators of innate defense and have been extensively studied in the elderly. The functional status of NK cells is the result of a balance between activating and inhibitory signals delivered by specific membrane receptors. Thus, NK cell killing of target cells requires not only the interaction of activating NK receptors with their ligands on the targets but also the lack of inhibitory signals initiated by the interaction of NK inhibitory receptors with target MHC class I molecules. Many NK receptors have been described to have a role in the recognition and regulation of cytotoxicity of tumor cells and virally infected cells (Bottino et al., 2006).

Cumulative evidence in the last two decades supports the importance of NK cell activity in maintaining good health during aging. Well-preserved NK cell cytotoxicity is observed in healthy elderly individuals and in centenarians, in particular in those with other criteria of healthy status such as physical fitness, independence to perform daily activities, or adequate cognitive function (Solana and Mariani, 2000). On the contrary, elderly individuals suffering from chronic diseases and frail elderly are characterized by lower NK cytotoxicity. Thus, low NK activity is associated with development of infections and death due to infection (Ogata et al., 2001), as well as with other medical disorders such as atherosclerosis (Bruunsgaard et al., 2001). Furthermore, high NK cell cytotoxicity is related to better health status and lower incidence of respiratory tract infections in elderly individuals, as well as better development of protective antibody titers in response to influenza vaccination (Mysliwska et al., 2004). Together, these results support the hypothesis that high NK cytotoxicity can be considered a biomarker of healthy aging and longevity, whereas low NK cytotoxicity is a predictor of morbidity and mortality due to infections (Figure 1). These studies could explain previous discrepancies on the effect of aging on NK cell cytotoxicity that did not consider the health status of the subjects studied and underscore the necessity of studies not only in healthy elderly individuals but also in nonhealthy or frail elderly, who have a higher risk of infection and related complications and represent the majority of elderly individuals.

The maintenance of NK cell activity in healthy elderly individuals is associated with an increased number of NK cells indicating that there is a decrease in cytotoxicity per NK cell in the aged, probably due to inefficient signal transduction (Solana and Mariani, 2000). Little is known about any changes in the expression and function of activating and inhibitory NK receptors in the elderly. However, NK cell activation mediated by CD16, the Fc γ RIII, seems unaffected by aging, indicating that NK activation and cytotoxic granule release remain intact (Solana and Mariani, 2000; Bruunsgaard et al., 2001; Lutz et al., 2005). Because the per-cell cytotoxicity against conventional NK targets is decreased in the elderly, the maintenance of CD16-mediated killing supports the view that either the expression or the functionality of other NK-activating receptors is defective in the elderly, or that there is overexpression of inhibitory receptors. NK cells from elderly individuals present an age-related increase in killer cell immunoglobulin-like receptor (KIR) expression and a reciprocal decrease in CD94-NKG2A expression, although the CD94-NKG2A inhibitory signaling pathway is intact (Lutz et al., 2005). The ability of IFN- α and IFN- β to enhance cytotoxicity of NK cells is also decreased with age, both in mice and humans. This defect could be related to the delay in clearance of virus after infection observed in aged mice, but this hypothesis requires further evaluation (Murasko and Jiang, 2005). In addition, the secretion of IFN- γ after stimulating purified NK cells with IL-2 also shows an early decrease, which can be overcome by prolonging the incubation time (Murasko and Jiang, 2005). NK cells from healthy elderly subjects retain the ability to synthesize chemokines and express the corresponding chemokine receptors. IL-12 or IL-2 can upregulate chemokine production although to a lesser extent than that observed in young subjects (Mariani et al., 2002). Therefore, these results collectively suggest that NK cells have an age-associated defect in their response to cytokines with a subsequent detriment in their capacity both to kill target cells and to synthesize cytokines and chemokines. Correlations between integrity of the NK system and health and longevity suggest that much more research should be focused on the age-related changes of NK cells with a view to preventing or reversing their compromised function.

Conclusions and Perspectives

Scientific and technical progress during the last few years has increased our knowledge of the innate immune response. Thus, the definition of new receptors on cells of the innate immune system, the advances in understanding the interactions among these cells to orchestrate adequate but not overzealous inflammatory and immune responses against pathogens, together with the advances in knowledge of signal transduction pathways and activation, compel additional efforts to further analyze how the aging process itself affects these aspects of innate immunity. The tools available at present, including proteomics and genomics, will allow us to identify the molecular mechanisms that contribute to the age-related dysfunction of the innate immune response and to initiate therapies to correct them.

Despite the maintenance of some innate immune functions in the healthy elderly, an increasing body of evidence indicates that aging has a negative effect on

the function of the innate immune system. Furthermore, innate immunity is severely diminished in nonhealthy and frail elderly, and this dysfunction contributes to the decline of the overall immune responses and to increased morbidity and mortality in the elderly due to infection. Thus, studies that analyze innate immunity in elderly individuals that are not affected by clinical conditions that could influence the immune system nor using drugs that could interfere with immunological parameters are able to provide information mainly on the intrinsic effect of aging on innate immunity. In contrast, those studies performed in nonhealthy or frail elderly are biased by the cumulative effect of extrinsic factors such as inflammatory diseases, chronic infections, cancer, malnutrition, or cognitive disorders. However, it is necessary to foster studies on innate immunity not only in healthy elderly individuals but also in nonhealthy and frail elderly, as these subjects are at higher risk of infection and related complications and represent the majority of elderly individuals. As innate immunity is the first line of defense against pathogens, boosting innate immunity in aged individuals may become an alternative strategy to fight infection and improve quality of life, provided that exacerbation of inflammatory conditions can be avoided.

The possibility to extend the immunological component of longitudinal investigations will help to understand the significance of alterations of the innate immune system as biomarkers of healthy aging and longevity. Longitudinal studies offer the only possibility in humans to track which changes in the innate immune system are really relevant to morbidity and mortality. Thus far, only certain aspects of adaptive immunity and parameters of inflammation have been considered in these exceptionally informative studies (Pawelec et al., 2005; Wikby et al., 2005), but to obtain a complete picture, assays of innate immunity must be included in these and other longitudinal studies in future.

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